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Blood parameter changes during stopover in a long-distance migratory shorebird, the bar-tailed godwit Limosa lapponica taymyrensis

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Bar-tailed godwits migrate from West African wintering sites to breeding areas in northern Russia with only one stopover. We compared hematocrit (Hct), blood hemoglobin concentration (Hb), and mean cell hemoglobin concentration (MCHb; a measure of the relative proportion of Hb in the cellular blood fraction) between arriving godwits lured to land 60 km short of the stopover site and godwits during subsequent refueling. The Hct and Hb of arriving godwits was low when compared to that of refueling birds. On the stopover site, Hct and Hb correlated positively with size-corrected body mass. In addition, Hb and MCHb reached peak levels in the last days of stopover. We explored the possibility of regenerative anemia in arriving godwits by comparing the fraction of reticulocytes (young red blood cells) between arriving and refueling birds. No differences were found. Therefore, we suggest that the increase in Hct, Hb, and MCHb during refueling is not in response to a severe anemic state at arrival. Rather, we suggest that the increase in blood parameters may anticipate the increased aerobic requirements of impending migratory flight and possibly satisfy heightened oxygen demands of the larger body mass of fattened birds. The Hct increase on the stopover site may also serve to buffer the red blood cell population against possible red blood cell breakdown during long-distance flight.

Many birds increase hematocrit (Hct) during migration (Banerjee and Banerjee 1977, DeGraw et al. 1979, Wingfield and Farner 1980, Thapliyal et al. 1982, Bergmann et al. 1994, Morton 1994). An increase in Hct typically implies an increase in red blood cell number, and an associated rise in oxygen-carrying capacity. To examine how blood parameters change in migrants during stopover, we examined Hct, hemoglobin concentration (Hb), and mean-cell hemoglobin concentration (MCHb) of bar-tailed godwits *Limosa lapponica taymyrensis* during arrival at a stopover site, and then during subsequent refueling.

Bar-tailed godwits winter in West Africa and breed in west and central Siberia (Drent and Piersma 1990, Piersma and Jukema 1990). Godwits execute a single refueling stop in the Wadden Sea of The Netherlands, Germany and Denmark (Piersma and Jukema 1990). Piersma et al. (1996) investigated changes in blood parameters of godwits refueling in the Wadden Sea and found that Hct was highest before the re-initiation of migratory flight. To further address how blood parameters change during stopover, we compared Hct, blood Hb, and MCHb between freshly arriving godwits that were lured to land 60 km short of the Wadden Sea and godwits caught during subsequent refueling. We predicted that Hct and Hb would be low in arriving birds, but would increase during refueling to reach peak levels in birds preparing to re-initiate flight. We also compared the percentage of reticulocytes (young red blood cells) between arriving and refueling birds. Reticulocytes indicate active red blood cell synthesis and are prevalent in cases of regenerative anemia (Ritchie et al. 1994, Campbell 1995). We predicted that refueling godwits would have a higher reticulocyte fraction than arriving birds, indicating elevated red blood cell synthesis in association with the Hct increase during stopover.

Methods

Subjects

We captured godwits at two sites along their migratory journey: (1) at the tail-end of a 4,500 km flight from West Africa to the Wadden Sea (52°32′N, 04°37′E), and (2) on the normal Wadden Sea stopover site (°03′N, 04°48′E). Birds at the tail-end of flight were intercepted in a dune reserve about 60 km short of the Wadden Sea (see Landys-Ciannelli et al. 2002). To lure flocks into this area we played recordings of bar-tailed godwit calls and displayed bird models. Arriving birds were cap-

tured with clap nets between April 29–May 9 1998, May 1–5 1999, and May 4–7 2000.

On the regular stopover area, refueling birds were captured with a large wind-driven pull-net, the "wilsternet" (Jukema et al. 2001) between May 19–30, 1995 (Piersma et al. 1996), May 13–22, 1998, May 15–21, 1999, and May 12–19, 2000.

Sampling

Blood was collected from the brachial wing vein into heparinized micro-hematocrit capillary tubes within 5 minutes of capture. Hb was estimated immediately after blood collection using the method described in Piersma et al. (1996). The remaining blood was centrifuged for ten minutes at $6\,900 \times g$. After centrifugation, Hct (packed red cell volume) in the capillary tubes was measured with a ruler as the percent cellular fraction of total blood volume. Because Hct is a measure of relative volume, Hct can vary as a result of either changes in the cellular or acellular blood fractions. In this study, we assumed that any changes in Hct were due to differences in the cellular blood fraction, because plasma volume in birds is largely resistant to change, even as a consequence of flight-incurred dehydration (Carmi et al. 1993). Hct and Hb were determined for birds captured in the years 1995 and 1998. MCHb was calculated according to: MCHb = Hb/Hct, and is a measure of Hb content relative to the cellular blood fraction.

Reticulocyte proportion was determined for arriving and refueling birds captured in the years 1999 and 2000. We prepared slides from collected blood by making blood films according to the method described in Campbell (1995). New methylene blue stain was added to dried blood films to test for presence of reticulocytes according to the method described in Christopher and Harvey (1992). Resulting wet mounts were examined under an oil immersion lens. We counted at least 500 red blood cells per slide to estimate reticulocyte proportion. Reticulocytes were identified by their relatively larger size and less condensed chromatin than that of mature erythrocytes, and the distinct ring of aggregated reticulum surrounding the cell nucleus (Campbell and Dein 1984, Campbell 1995).

Godwits were banded, measured, and sexed on the basis of plumage and size characteristics (Piersma and Jukema 1990). Even though moult is suspended before initiation of migratory flight from West African wintering grounds, high quality birds are able to reinitiate contour feather moult on the Wadden Sea stopover site (Piersma and Jukema 1993). We scored moult on the stopover site as either present or absent according the method presented in Piersma and Jukema (1993). Birds that had completed moult during their stay in the Wadden Sea were still characterized as moulting birds, and were recognizable due to traces of waxy sheaths at feather bases.

Data analysis

Because godwits are sexually dimorphic (Piersma and Jukema 1990), we corrected body mass for sex-related size differences by calculating mass residuals for all birds according to the relationship between body mass and wing length (as described in Landys-Ciannelli et al. 2002). Mass residuals were back-transformed to a normalized female size-corrected body mass estimate (in grams) for use in figures.

Hct and reticulocyte percentage were arcsin square root transformed for use in statistical tests. Stopover was divided into 3 periods according to calendar day: early refueling (May 13–16), mid-refueling (May 19–25), and pre-departure (May 29–30; birds typically depart the Wadden Sea stopover site in late May (Piersma and Jukema 1990, Green et al. 2002)). The variable: sex was excluded from the models considered here because Hct, Hb, and MCHb did not differ between sexes (Mann-Whitney Rank Sum Tests: $P = 0.731$ and $P = 0.487$, and Student's t-test: $P = 0.536$, respectively). Bonferroni-adjusted α-values were used for statistical tests in which the same data were analyzed twice, i.e., variables in these tests were considered significant only if their associated P-values were less than 0.025 (Sokal and Rohlf 1995). All data in the results are presented as averages $\pm$ SD.

Results

Hct and Hb were significantly lower in arriving godwits than in refueling birds (Student's t-test: $P < 0.001$ and Mann-Whitney Rank Sum test: $P < 0.001$, respectively; see Fig. 1). The Hct of arriving and refueling godwits was $0.47 \pm 0.04\%$ and $0.51 \pm 0.04\%$, respectively. The Hb of arriving and refueling birds was 15.4 ± 1.5 g/100 ml and 17.6 ± 2.7 g/100 ml, respectively. MCHb did not differ between arriving and refueling birds (Mann-Whitney Rank Sum test: $P = 0.097$).

During stopover, Hct and Hb correlated positively with size-corrected body mass (see Fig. 1 and Table 1). Also, Hb and MCHb changed with refueling period (see Fig. 2 and Table 1), i.e., Hb and MCHb were highest in birds preparing to depart the stopover site (Tukey post-hoc test: $P < 0.01$), but were similarly low in early and mid-refueling birds (Tukey post-hoc test: $P > 0.10$). Hct did not change with refueling period (see Table 1).

Reticulocyte percentage was not significantly different between arriving and refueling godwits ($4.5 \pm 4.5\%$ and $3.8 \pm 3.7\%$, respectively; Student's t-test: $P = 0.722$).

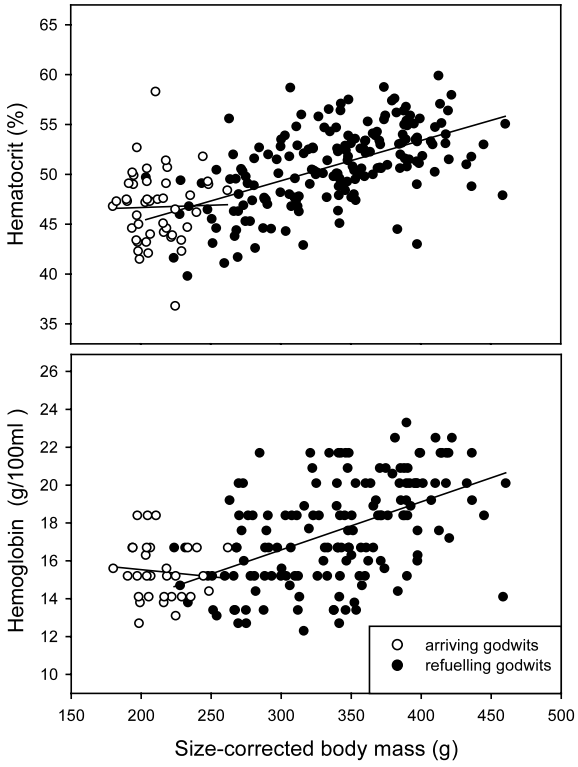

Fig. 1. Blood parameters as a function of size-corrected body mass in arriving and in refueling godwits. Hct and Hb were lower in arriving godwits than in refueling birds. Hct and Hb increased with size-corrected body mass throughout the period of stopover. Even though slopes between arriving and refueling godwits were not compared, a slope is drawn through data from arriving godwits for purposes of presentation.

Discussion

In comparison to refueling birds, godwits captured during arrival to the Wadden Sea stopover site displayed low Hct and Hb. During subsequent refueling, Hct and Hb increased with size-corrected body mass. In addition, godwits displayed higher Hb and MCHb toward the end of the stopover period, i.e., before departure to the breeding grounds.

Table 1. Results of ANCOVA tests on Hct (%), Hb (g/100 ml) and MCHb (g/100 ml) of godwits refueling in the Wadden Sea. Period of stopover and presence/absence of moult were included as factors. Mass residuals were included as a covariate after the homogeneity of slopes between regression lines was verified.

Factor/variable	Hematocrit P-value	Hemoglobin P-value	MCHb P-value
Period	0.165	<0.001	0.002
Moult	0.546	0.208	0.314
Period*Moult	0.688	0.472	0.543
Mass Residuals	<0.001	0.001	0.709
N	181	154	152
R^2	0.336	0.397	0.200

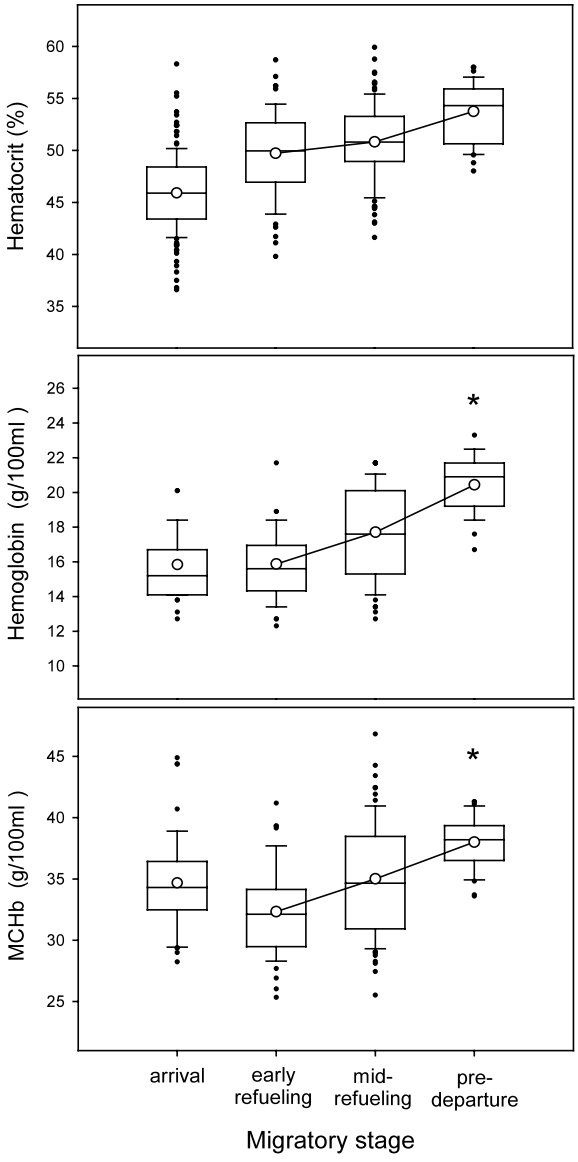

Fig. 2. Blood parameters of godwits according to stopover period: early refueling, mid-refueling, and pre-departure. Hb and MCHb were higher in birds preparing to depart the stopover site than in early or mid-refueling birds. Hct did not change with refueling period. Even though arriving godwits were not compared to refueling birds, arriving godwits are included in the figure for purposes of presentation.

Because the body mass of refueling godwits is an indication of their readiness to re-initiate flight (Green et al. 2002), the correlation between size-corrected body mass, and Hct and Hb, suggests that red blood cell number increases as birds prepare to depart from the Wadden Sea. An increase in red blood cell number during refueling may alleviate increased aerobic requirements during flight. However, the correlation between body mass and blood parameters may also signify that birds with a larger tissue mass have greater

oxygen requirements, to which they respond by increasing red blood cell number. Studies on obese animals verify that a larger body mass is associated with significantly higher Hct (Yen et al. 1970, Failla et al. 1988, Micozzi et al. 1989).

The rise in Hb and MCHb in the final period of stopover suggests that birds increase the proportion of Hb per red blood cell immediately before flight in a final effort to elevate blood oxygen carrying-capacity. A rise in MCHb reduces the need to increase red blood cell number and thus prevents high blood viscosity and inefficient circulation (Smith et al. 2000).

Because godwits were not sampled after departure from the stopover site, we were unable to determine whether blood parameters change during the course of migratory flight. In light of the fact that Hct and Hb show a progressive decrease with flight distance in homing pigeons (Riera et al. 1983, Bordel and Haase 1993), migratory flight may result in an exhaustion of red blood cells, possibly due to activity-associated stresses (Mairbaurl et al., 1983, Reinhart et al. 1983). Therefore, in addition to supporting aerobic needs during flight, the Hct increase in refueling birds may anticipate red blood cell breakdown and serve to buffer red blood cell number.

We compared the proportion of reticulocytes between birds arriving to the Wadden Sea and birds refueling on the stopover site to test for the presence of regenerative anemia during stopover. Reticulocyte proportion was similarly low in arriving and in refueling birds (i.e., it was below the 10% typical of certain avian groups; Campbell 1995), suggesting that the increase in blood parameters during stopover was not in response to a severe anemic state at arrival.

In conclusion, godwits arrived to the Wadden Sea stopover site after a 4 500 km flight with relatively low Hct and Hb. During refueling, Hct and Hb increased with size-corrected body mass. In addition, Hb and MCHb were highest in the final period of stopover, suggesting an increase in oxygen carrying-capacity not only as a function of increased red blood cell number but also due to a greater proportion of Hb per red blood cell. Because reticulocyte proportion was low at arrival and remained low throughout stopover (indicating the absence of a regenerative anemia), the increase in Hct, Hb, and MCHb during refueling is unlikely to be in response to a severe anemia. Rather, we suggest that the elevated blood parameters in refueling birds may anticipate increased aerobic requirements during impending flight and also satisfy the heightened oxygen demands of a larger tissue mass. Elevated Hct in departing birds may also serve to buffer the red blood cell population against possible red blood cell breakdown during flight.

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Sex-biased nestling mortality in the Montagu's harrier Circus pygargus

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I evaluate causes and patterns of nestling mortality in a sexually dimorphic species, the Montagu's harrier *Circus pygargus*, and their relationship with sex and condition. Starvation was apparently the main reason for nestling death. Condition of birds that died was lower than those that survived. Both probability of nestling death and the proportion of nestlings that died within a brood increased with the number of hatched nestlings in a brood, and with increasing hatching date. For the nestlings that died after being sexed, when controlling for brood effects, probability of death was significantly related to nestling sex, with smaller males having a higher probability of dying. The probability of nestling death if hatched late in the season was relatively greater for males than for females. There was also a significant interaction between sex and hatching date on nestling condition: the decline in condition if hatched late in the season was steeper for males than for females. Males did not have a higher probability of death when having more sisters: neither the probability of brood reduction nor the proportion of nestlings that died were significantly related to within-brood sex ratio. Results suggest that mortality may partly result from sibling competition: females, being the larger sex, might be better able to compete for food within a brood than their male siblings. Additionally, smaller males may be less able to recover from periods of declining body weight.

Males and females differ physiologically and behaviourally, so may be differentially susceptible to sources of mortality. In vertebrates, the juvenile stage is a period of heavy mortality, both before and after the termination of parental care. Whether mortality in juveniles is sex-biased is important because sex-biased mortality could be a cause for biased sex ratios at the end of parental care, or among adults (Trivers and Willard 1973, Breitwisch 1989).

Body size can influence survival in both juveniles and adults. Therefore, in animals with sexual size dimorphism, sex biased mortality may occur due to the different size, and this has indeed been found in many bird and mammal species (Breitwisch 1989, Clutton-Brock 1991). However, the direction of the bias may depend on the cause and the timing of death.

In altricial birds, before the termination of parental care, juveniles depend on food provided by their parents. The larger sex is usually more expensive to raise, shows higher growth and/or metabolic rates, and thus overall higher nutritional requirements (Clutton-Brock et al. 1985, Slagsvold et al. 1986, Teather and Weatherhead 1988, Griffiths 1992, Anderson et al. 1993a, Bennett et al. 1995, Riedstra et al. 1998, but see Torres and Drummond 1999). As a result, biased mortality in juveniles towards the larger sex is relatively common in birds and mammals, and is associated with differences in parental investment in both sexes. In addition, the bigger sex may show a greater susceptibility to food shortages associated with their faster growth rates and/or increased nutritional requirements (Clutton-Brock et